PROJECT FINAL REPORT

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4.1 Final publishable summary report

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Executive summary

The general objective of ETHERPATHS was to build a suite of tools that will afford studies of foods modulating lipid metabolism, from systemic to cellular levels. The theme of the project was the control of systemic lipid homeostasis and the role that nutrition may play in its maintenance, improvement or dysregulation. In particular, we studied the relevance of plasmalogens, which are enriched in n-3 fatty acids and are already known to play an important role in human health as a factor involved in aging, obesity, diabetes, and diseases of the central nervous system.

As part of the ETHERPATHS platform for nutritional systems biology, *in vitro* studies were supporting the studies *in vivo* as well as the clinical intervention study. For example, *in vitro* colon model was applied to ten substrates used in the diets of human intervention conducted in the project, and predicted the compliance markers from polyphenol-rich diet. These markers were later validated in the nutritional intervention study. The metabolite profiles of samples obtained from the colon model were analysed. The purified faecal water extracts were prepared from these studies and applied in hepatocyte and adipocyte interventions. The *in silico* strategies to complement the *in vitro* studies included (1) modifications of the IsoDyn model, a model of central carbon metabolism, and (2) development of a model of palmitate conversion into VLDL in hepatocytes.

The main focus of *in vivo* studies was on generation and characterization of the plasmalogen deficient mice (*Gnpat* KO mouse). A model of *Gnpat* depletion and phenotyping showed that *Gnpat* depletion has an impact in the overall energy homeostasis. Studies were also conducted to investigate how the gut microbiota affects host lipid metabolism by comparing serum, white adipose tissue, and liver lipidomes in germ free and conventionally raised mice under different diets.

The four diets were developed differing for n-3 fatty acid and polyphenol content, which were used in the ETHERPATHS nutritional intervention study in humans: (A) Control diet low in n-3 fatty acids and polyphenols; (B) Diet rich in n-3 fatty acids and low in polyphenols; (C) Diet rich in polyphenols and low in n-3 fatty acids; (D) diet rich in n-3 fatty acids and polyphenols. The schedule for type, time and modalities of sampling, storing and shipping for the blood, urine, faeces and adipose tissue samples to be analysed at the end of the intervention periods has been defined. Dietary compliance was optimal. The diets were found to have distinct effects on the lipid profiles as well as on gut microbial composition.

New computational and analytical tools were developed to support the nutritional systems biology platform. Metabolomics data processing software MZmine 2 was released and optimized to detect stable isotope tracer signals. A method for use of heavy water as tracer for *de novo* lipogenesis has been implemented. The protocol for new kinetic studies has been developed. New efficient methodologies for the characterization of lipid and small polar metabolite profiles in biological samples were set up. For the metabolite identification, mass spectra libraries utilising the accurate mass and high resolution mass spectrometry were constructed for different sample matrices, including serum and tissue. New data analysis methods for the treatment of the metabolomics data were developed and released.

The Web platform was set up which integrates tools for experimental data management and a Systems Biology Markup Language (SBML) model designer and manager. Also, the Meta-Model of lipid spillover in whole organism was developed and integrated with the Web platform. Kinetic model of fatty acid metabolism in hepatocyte was added to Meta-Model. Its adequacy was demonstrated through virtual experiments as well as by generating and validating the predictions based on experimental data generated in the project.

During the last period of the project, also the dissemination and exploitation activities have been intensifying. The ETHERPATHS platform has been presented at multiple conferences (e.g., special ETHERPATHS session at NuGOweek 2012). Also a book has been released ‘A Systems Biology Approach to Study Metabolic Syndrome’ (Springer Verlag, ISBN 978-3-319-01007-6, eds. Matej Orešič and Antonio Vidal-Puig), which includes among others 10 contributions from the ETHERPATHS participants.

Project context and objectives

The collaborative project **ETHERPATHS** specifically addressed the call *FP7 Cooperation Work Programme: KBBE-2007-2-2-08: Systems Biology and bioanalytical tools for nutrition research.*

Understanding of living organisms in the context of coordinated gene and molecular function and translation of this knowledge to improve human health is a great challenge and certainly one of the central aims of medical systems biology. The interface between biological systems and nutritional factors represents the next level of complexity involving interactions between nutrients, host metabolism and gut microbiota that still have to be understood. *Nutritional systems biology* thus requires bridging across multiple levels and concepts, *e.g.*, cellular–organismal, host–microbial, short-term–long-term effects. To face such a complex task, it is essential that the systems biology tools are built by combining multiple experimental and theoretical approaches, based on concrete problems and clearly defined questions.

The **theme** of this collaborative project was the control of systemic lipid homeostasis and the role that nutrition may play in its maintenance, improvement or dysregulation. In particular, we will study the relevance of *plasmalogens*, which are enriched in n-3 fatty acids and are already known to play an important role in human health as a factor involved in aging, obesity, diabetes, and diseases of the central nervous system. The **concept** is that the study of the regulation of systemic lipid homeostasis in the context of nutrition is an excellent case study, with the sort of clearly definable questions needed to build the tools for nutritional systems biology.

The **overall objective** of the project was to build a suite of tools that will afford studies of foods modulating lipid metabolism, from cellular to systemic levels.

The expected **impact** is the development of new informatics and analytical tools to bridge data across *in vitro*, *in vivo*, and clinical levels by using cellular, tissue level, and physiological models; and to gain new knowledge on how various dietary components, such as n-3 fatty acids and dietary polyphenols, as well as gut microbial composition modulate lipid homeostasis beneficially for human health.

The main objective of ETHERPATHS was met by combinations of wet-lab and dry-lab strategies at *in vitro*, *in vivo*, and clinical levels, divided into six specific objectives:

O1.Provide *in vitro* and *in silico* platforms for studies of gut microbiota in the context of nutritional interventions.

ETHERPATHS will focus on linking metabolism of fatty acid supplements with those containing polyphenols, which are converted by gut microbiota and circulated in entero-hepatic-circulation and plasma.

O2.Generate and characterize a plasmalogen-deficient mouse model in the context of nutritional interventions, specific lipid metabolism modulation, and gut microbiota variation.

ETHERPATHS will identify the mechanistic links between nutritional interventions involving fatty acid supplements and polyphenols, and **systemic plasmalogen status**. New pathway reconstruction tools will be developed for that purpose, which will complement and link to the modelling tools and results from *in vitro* studies.

O3.Provide bioanalytical and dynamic modelling tools for studies of systemic lipid metabolism in the context of nutritional interventions.

ETHERPATHS will use samples and data from an ongoing clinical trial, as well as conduct a new trial involving specific nutritional interventions. New physiological models of lipid metabolism will be developed based on the data, which will include modelling of lipids in very low–density lipoproteins (VLDL) as well as in chylomicron particles, thus establishing a link with the intestinal lipid metabolism.

O4.Provide software and modelling tools for integration of computational models and data across multiple organism levels.

ETHARPATHS will combine modelling tools used to reach objectives 1-3, in a single Systems Biology Markup Language (SBML)-compliant software platform. Using advanced and emerging integrative bioinformatics strategies such as *conceptual spaces*, the data and model entities will be linked to established knowledge repositories and databases. New theoretical models will be developed aiming to investigate control and dependencies between different levels, *e.g.*, gut microbiota and its metabolites, tissues, and systemic lipid metabolism.

O5.Design and perform nutritional interventions, including food components that are metabolized in the colon, to define their specific alterations in lipid homeostasis.

Evaluate the effects of diets rich in ω-3 fatty acids and polyphenols on lipid metabolism. Investigate possible mechanisms of their effects.

O6.Develop a high-throughput mass spectrometry-based platform for sensitive profiling of lipids.

ETHERPATHS will build on most recent developments in nano-electrospray ionization (nanoESI) microfluidistic chip and electrocapture technologies to develop highly sensitive and quantitative platforms for global profiling of lipids and hydrophilic metabolites.

Main project results and foregrounds

The research progressed in all six workpackages (WPs, shown in Figure 1 below), corresponding to six specific objectives of the project.

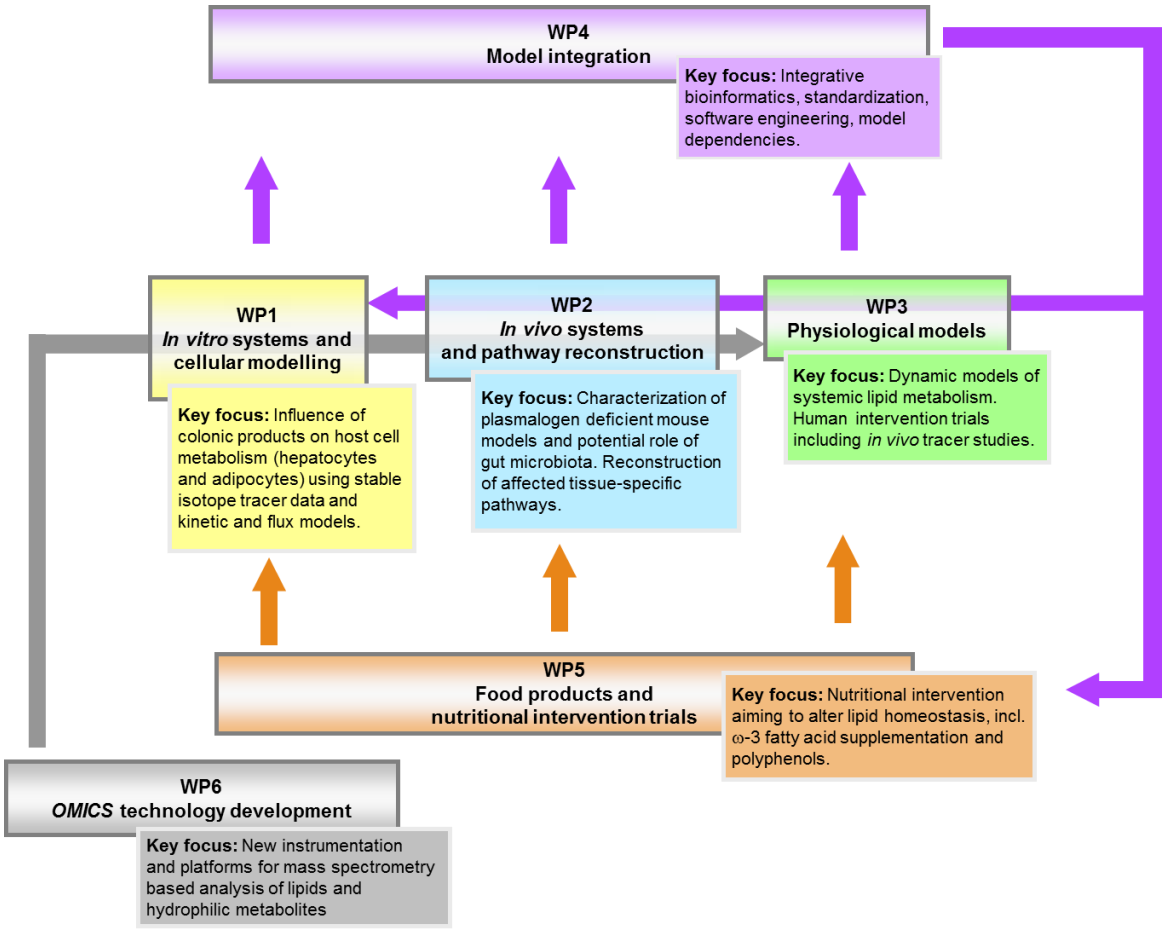


Figure 1 ETHERPATHS project structure.

WP1

In **WP1**, during the first period (months 1-18), *in vitro* colon model was applied to eight substrates used in the diets of ETHERPATHS human intervention study, which was performed in Naples. Purification techniques to isolate faecal microbiota from faecal water were tested and hydrophobic PTFE membrane was selected. Metabolite profiles were analysed. Statistical analysis methods for quantitative targeted analysis and semi-quantitative metabolomics were established and applied to all data and to red wine, respectively. In the 2nd period (months 19-36), semi-quantitatve metabolomics was applied to those selected foods, which showed formation of known microbial metabolites of phenolic compounds, namely green tea, orange, rocket salad. Furthermore during the 2nd period additional substrates (spinach, artichoke and coffee) were subjected to the colon model for identification of their food-related intestinal metabolome. Exctracts fro cell assays were prepared, including converted green tea (with microbiota), non-converted green tea (in buffer, no microbiota) and microbial control (faecal microbiota, no green tea) after oincubation of 6 hours. Experimental data from VTT (production of polyphenols and other metabolites by colonic microbiota) were processed at ISBSPb and kinetics parameters for meta-model were estimated. Using developed version of meta-model the preliminary predictions of blood dynamics of different metabolites were made (Figure 2).

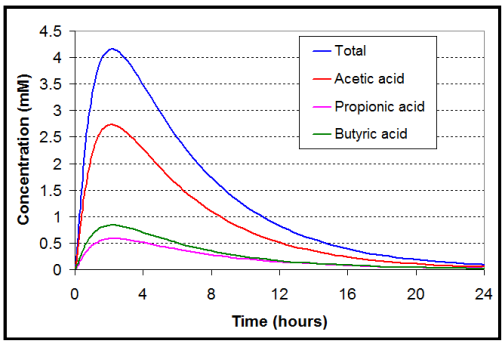


Figure 2 Blood dynamics of short chain fatty acids after single consumption of green tea. Model prediction based on parameters fitted with VTT data (clearance parameters are arbitrary).

During the 3rd and last reporting period (months 37-54), the prediction of average human urinary excretion of quantitative metabolite profiles was calculated from the colon model data on the basis of metabolite profiles at 6 hour time point, the fresh weights and portions and frequency of the intake of polyphenol rich foods. The data showed the same significant metabolites as the most ubundant hydroxylated phenylpropionic, -acetic and benzoic acids, indicated as major metabolites in the colon model. The compliance was also indicated by 7-day food records and the results were in agreement. Further conversion of microbial metabolites from green tea in hepatocytes also confirmed the role of liver in the formation of the metabolic pool and filled the gap between colon model and human urinary excretion of polyphenol metabolites.

During the 1st period, the experiments related to hepatocytes and adipocytes were initiated by protocol modifications for hepatocytes and adipocytes. Cultivation of hepatocytes has started and the first experiments with pre-adipocytes and adipocytes have been performed to design the future incubation conditions. Purified faecal water extracts were prepared from selected colon model samples and corresponding controls for testing their purity in the hepatocyte interventions. Purification of microbial extract was adjusted. The generation of an *in vitro* model of plasmalogen depletion was conducted to investigate the effects of plasmalogens and dietary components in adipose tissue using loss of function strategies, *i.e.*, establishment of *Gnpat* knockdown 3T3L1 white preadipocyte cell lines.

During the 2nd period we found that benzoic acid derivatives were the main metabolites from incubation of hepatocytes with colonic phenylpropionic acid-rich extracts and that it is feasible to study oleic acid intervention to show lipoprotein secretion from human primary hepatocytes. In the 3rd reporting period the hepatic metabolism of green tea and its microbial metabolites were confirmed using human primary hepatocytes. 3,4-Dihydroxypropionic acid, 3-hydroxybenzoic acid, ferulic acid and hippuric acid were confirmed the major hepatic metabolites from converted green tea. We also found that McA-RH777 hepatocyte cells produced glucose, lactate and glutamine without an apparent consumption of amino acids. It would suggest that this cell line has another source of carbons that is able to contribute to the central carbon metabolism. The isotopologue distributions obtained after the 8 hours of incubation with the tracer suggest that these cells use glucose from the medium to recycle glycogen while they produce new glucose molecules from other carbon sources. However, other metabolites such as lactate, amino acids (Figure 3) and TCA cycle intermediates seem to be mainly produced by other carbon source/s.

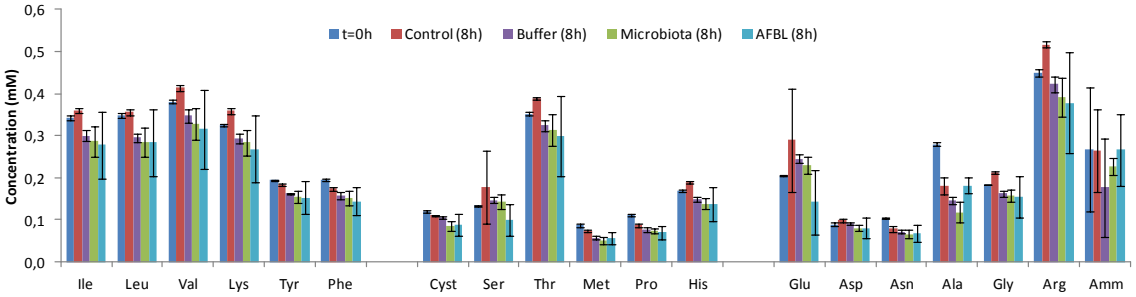


Figure 3 Concentrations of amino acids in the incubation medium of McA-RH7777 cells.

The three tested colonic extracts have different effects in the McA-RH7777 central carbon metabolism. Despite the lack of modelling of the data, a different distribution in some of the central carbon metabolic pathways is expected, especially in those involving pentose phosphate pathway and TCA cycle. The highest metabolic effects were induced by the colonic extracts containing fermented and non-fermented green tea polyphenols. Moreover, their effects in McA-RH7777 cells were different, confirming that the fermentation is important for the metabolic effects.

During the 2nd period, we found:

1. *PUFA intervention.* The metabolic disturbances associated to the lack of plasmalogens (eg. lipid accumulation, markers of adipogenesis and lipogenesis) are directly related to the depletion of that specific subset of lipids or some products of their metabolization. No dietary or pharmacological interventions other than metabolites aimed to rescue plasmalogens levels "per se" totally recovered the phenotype but only improved some partial features. Globally considered our data indicate that plasmalogens (ether lipids) play a *unique* role in the differentiation programme of the adipocytes.
2. *Effects of extracts derived from green tea pre- and post-colonic fermentation on cell cultured adipocytes.* Globally considered, our results suggest that all three green tea experimental groups (Green tea with microbiota, Green tea in buffer and Faecal control) tested impacted at different degrees the metabolism of the 3t3l1 line after acute exposition. Indeed, increased lipogenic capacity as well as increased catabolism of lipids (lipolysis and fatty acid oxidation programme) and possibly, increase in glucose uptake is a common hallmark in response to these treatments.

During the 3rd period, we implemented the data regarding the metabolic impact of non-converted or converted green tea of the microbial control (no green tea) in adipocytes. These results demonstrated that those compounds affect glycolysis, lipolysis and mitochondrial respiration as well as insulin signaling cascade. The identification of the single molecules or combination of molecules from the different extracts responsible for such effects would be essential to explore and exploit any potential therapeutic use of those natural compounds in future. The other approach is that different extracts from the microbial conversion of different foods would be tested using varying time lengths and concentrations. The most important difference is to detect whether the pre- or post-colonic extracts are active and also take into account the hepatic metabolism of the non-converted food extracts.

During the 2nd period preliminary results showed little disturbance of the main adipocyte metabolism, suggesting a subtle effect of the colonic extracts studied at the concentrations used. During the last reporting period, converted Green tea extracts caused a state in the 3T3-L1 adipocytes, in which the cells required less reductive power. The changes were subtle, which also indicated that the adipocytes were not affected harmfully.

During the 1st period, the IsoDyn model for metabolic modelling based on 13C tracer data was also modified. The draft model of palmitate conversion into VLDL in hepatocyte was constructed as a link between central carbon metabolism model and the model of systemic lipid metabolism. Draft version of the model was updated to describe three fatty acids (palmitate, stearate, alpha-linolenic acid) conversion into VLDL particle.

During the 2nd period, methodology was implemented in IsoDyn software which can reveal compartmental structure and metabolic flux distribution from the distribution of ^{13}C isotopomers measured in the products of cells incubated with ^{13}C labeled substrates. The application of this methodology to the analysis of ^{13}C isotopomer distributions measured in metabolites of isolated liver cells revealed a separate compartment of hexose phosphates related with substrate channeling in glycogen metabolism. This analysis provided the distribution of metabolic fluxes in central carbohydrate metabolism of hepatocytes incubated with ^{13}C labeled glucose, and revealed the changes of fluxes in hepatocytes that were induced by addition of other sources of carbon in the incubation media.

A metabolic model for the analysis of flux distribution in adipocytes has been constructed. Simulation of ^{13}C isotopomer distribution with Isodyn revealed greatly increased fluxes from Krebs cycle and biosynthetic pathways in adipocytes compared to their undifferentiated precursors (Figure 4).

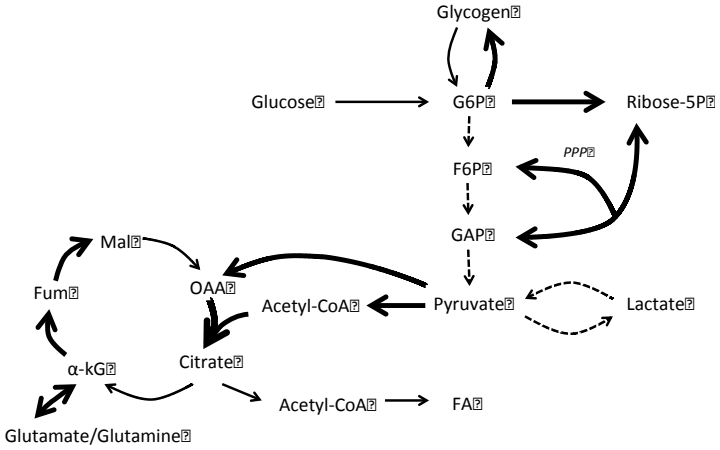


Figure 4 Summary of data from the ISODYN fitting. The bold arrows indicate fluxes that increase, and the dotted arrows are fluxes that are lower than in day 1.

All these analyses indicate that the tested polyphenol- or their microbial metabolite-enriched extracts do not affect hepatocyte metabolism, while the converted and non-converted polyphenols of green tea affect glycogen and glycolysis futile recycling in adipocytes.

During the 1st period, NorayBio started to prepare the design the DDBB (database) and the software structure during the 1st period and data of the first food related metabolite profiles were sent to NorayBio to facilitate the construction of the database. ISBSPb obtained experimental data from VTT (production of polyphenols and other metabolites by colonic microbiota) and the data were processed and kinetics parameters for meta-model were estimated. Using developed version of meta-model the preliminary predictions of blood dynamics of different metabolites were made.

During the second period NorayBio has coordinated the data collection among WP1 partners that was then used to feed the Meta-Model in WP4 (metabolite profiles, flux analysis data). Individual models were requested too to build the Meta-model. The requirements for the kind of models and data needed were studied and this information were sent to the partners.

Data files and format examples were used to test the performance of the Web platform.

During the 3rd reporting period NorayBio and ISBSPB applied their software and modelling tools to model the effects of microbial metabolome on hepatocytes and adipocytes optimized and validated them with the experimental data obtained in the project.

WP2

A pilot nutritional intervention was performed in ob/ob mouse models that suggests alkylglycerol (precursor of plasmalogens) treatment has a beneficial impact on serum triglyceride, free fatty acid and ketone body levels. This is accompanied by mild increases in genes involved in lipid oxidation in highly oxidative tissues such as heart and liver. In addition, lipidomic analysis also revealed remodelling in the lipid profile of key organs, including an increase in ether-lipid species. This supports the use of alkylglycerols as strategy to replenish ether-lipid pool.

University of Cambridge (UCAM) team also obtained a model of *Gnpat* (key gene in ether lipid synthesis) depletion (a hypomorphic) and started the metabolic phenotyping of the mice fed chow diet. Our data up to date show *Gnpat* hypomorphic mice are more glucose tolerant than the wildtype counterparts. Blood biochemistry revealed a substantial increase in ketone bodies. This latter result is in line with the gene profiling in liver increased expression of genes involved in fatty acid oxidation and ketone bodies production. Interestingly, skeletal muscle and brown adipose tissue also showed

and increase in fatty acid uptake and oxidation as well as increase in markers related to mitochondrial biogenesis.

VTT performed lipidomic analysis of different tissues of the *gnpat* C57BL6 ko mouse vs. wt littermates and confirmed the depletion of etherlipids was globally exacerbated in comparison to the mix background 129Ola/C57BL6. A new pathway reconstruction methodology was developed for understanding cellular lipid metabolism that can be applied to the in vivo profiling. Methodology was demonstrated in lipidomics data from liver tissue in plasmalogen-depleted mouse models but the methodology is flexible for any tissue data.

All in all, the data in this WP suggest that *Gnpat* depletion has an impact in the overall energy homeostasis.

University of Gothenburg team (UGOT; Fredrik Bäckhed) has investigated how the gut microbiota affects host lipid metabolism by comparing serum, white adipose tissue, and liver lipidomes in CONV-R and GF mice. They demonstrated that the microbiota modified a number of lipid classes in the serum, adipose tissue and liver, with its most dramatic effect on triglyceride and phosphatidylcholine species (Figure 5).

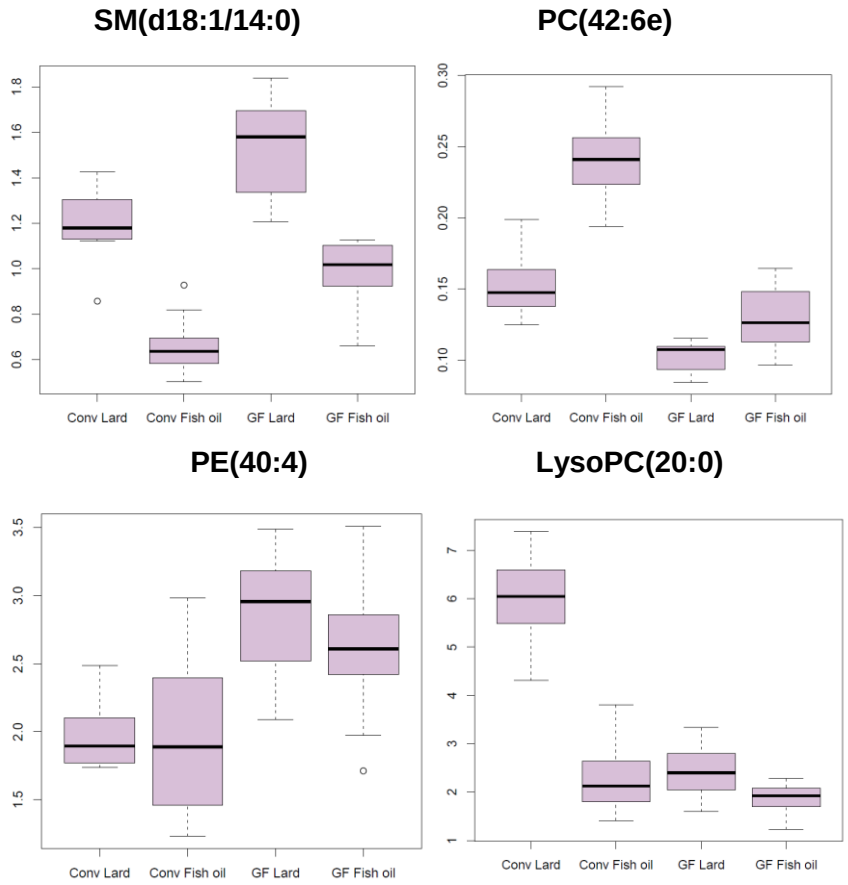


Figure 5 Examples of serum lipids regulated by gut microbiota.

WP3

UGOT (Jan Boren) has developed a new protocol for kinetic studies (Figure 6) and the outline of the model has been set.

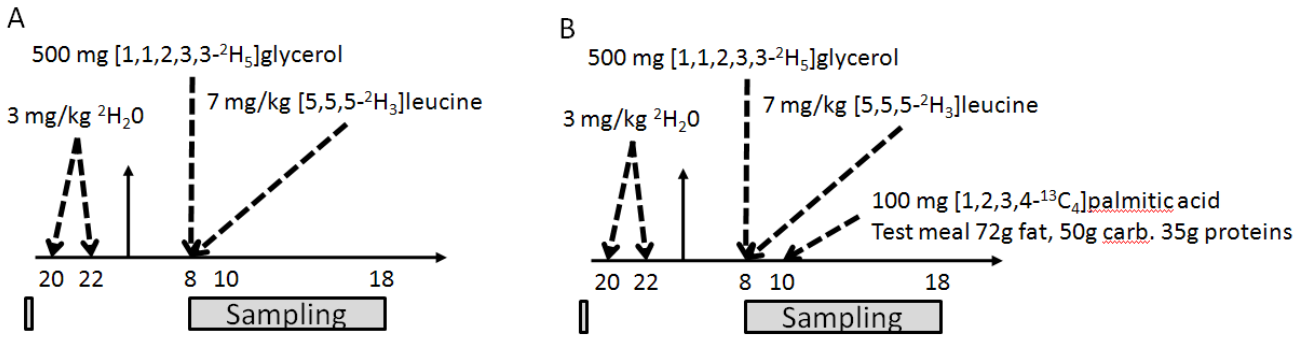


Figure 6 Outline of the two kinetic studies.

In the human kinetic studies, main core and surface lipids of VLDL1 and VLDL2 lipoproteins showed expected results with core lipids (TG and DAG) being 8-fold higher in VLDL1 than VLDL2 and surface lipids being about 2-fold higher. In contrast the surface lipid PE was markedly enriched (i.e more than two fold) in VLDL1. This may reflect a different site of maturation for VLDL1 than for VLDL2. This is in line with our results showing differential regulation of VLDL1 and VLDL2 secretion both in vitro and in vivo

The nutritional intervention showed altered levels in glycerophospholipids, mainly PC and PE, due to the high n-3 fatty acid diets (with or without a high content in polyphenols), while exclusive polyphenol modification caused only minor changes in the HDL fraction profile.

In the mouse lipoproteins analysis, lipidomic profiling of plasma HDL fractions showed that the depletion of ether phospholipids in *Gnpat* KO mice could be modulated by diet. Ether-lipids could partly be replenished with dietary n-3 fatty acids.

NorayBio has coordinated the data collection among WP3 partners that was then used to build the Meta-Model. The requirements for the kind of models and data needed were studied and this information was sent to the partners. Data files and format examples were used to test the performance of the Web platform. The physiological module of the Meta-Model (WP4) was updated using experimental data from WP3 and with published data. This was implemented in the Circulator software package (ISBSPb).

WP4

First 18 months of the project has been a very intense period in WP4. Outstanding results have been obtained at both main tasks of the WP: “Development of the software platform”, and “Development and implementation of the systemic Meta-Model”. Regarding the first one, a first version of the Web platform has been developed, which integrated tools for experimental data management and a SBML model designer and manager. The platform will also gives access to the Meta-Model server (MMS). Interface and communication to the MMS has been also defined. Regarding the second objective, the draft version of the Meta-Model of lipid spillover in whole organism was developed. Kinetic model of FAs metabolism in hepatocyte was added to Meta-Model. Its adequacy has been initially demonstrated through virtual experiments.

In the 2nd period, the development focus was on 1) new informatics module that gives access to the Metamodel Server as well as improvement and validation of the existing ones and 2) Systemic Meta-Model. Regarding the first one, a new version of the platform was released. Improvements and validation works were carried out for Experiments manager and SBML Models Designer modules. Meta-Model Server module was implemented based on the previous requirements analysis. Regarding the second one, inputs and outputs of the mathematical models have been interconnected with corresponding outputs and inputs of meta-model of lipid spillover in whole organism. The Meta-Model of lipid spillover in whole organism was updated too.

In the 3rd reporting period, NorayBio finished the web platform to allow data interchange and to make and manage simulations against the Meta-Model Server and their results (Figure 7). It has

been programmed in such a way that will be easily updated in case new processes are implemented in the future in the Meta-Model Server.

The University of Barcelona team (UB) released two models: model of adipocyte central carbon metabolism and model of hepatocyte central carbon metabolism, both implemented in SBML and adjusted with IsoDyn software. ISBSPb implemented the final version of meta-model that integrates literature available experimental data and reproduces some of experimental data obtained by other participants of project was developed. This version was used for optimization and validation of meta-model server and GUI.

Unlike other software platforms, the software platform released in WP4 allows the joint study of digestive, lymphatic and circulatory systems in humans and aims to become a new reference tool for the research on lipids nutrition.

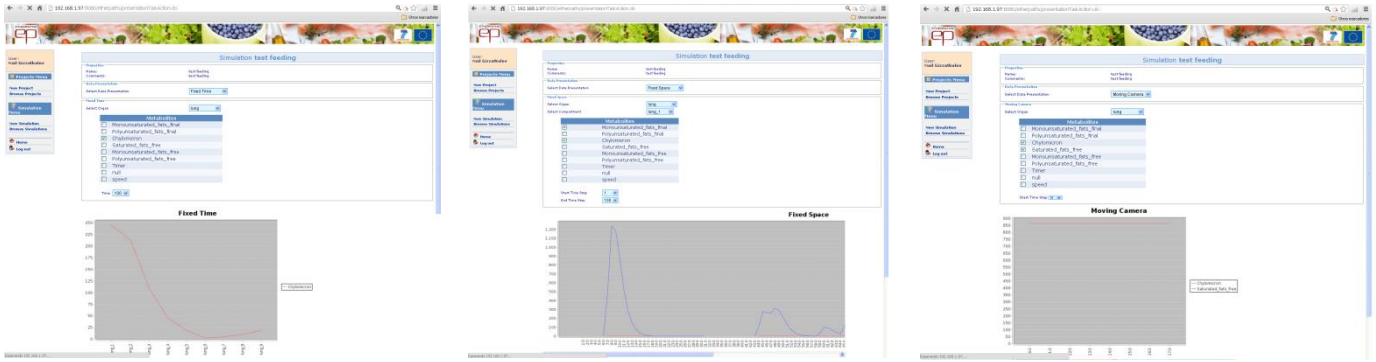


Figure 7 Graphical representation of the three modes in Meta-Model Server module. From left to right: Fixed Time, Fixed Space and Moving Camera.

WP5

The four diets have been developed differing for -3 fatty acids and polyphenols content to be used in the ETHERPATHS nutritional intervention study in humans: (A) Control diet low in n-3 fatty acids and polyphenols; (B) Diet rich in n-3 fatty acids and low in polyphenols; (C) Diet rich in polyphenols and low in n-3 fatty acids; (D) diet rich in n-3 fatty acids and polyphenols.

Beside the different content in n-3 fatty acids and polyphenols, the four diets are similar for all the other characteristics, including macronutrient composition and content of micronutrient that could possibly affect outcomes, especially for their antioxidant properties.

Polyphenol-rich foods were subjected to the colon model for identification of the microbial metabolites of polyphenols in WP1. Acceptability of the foods identified for the different dietary approaches has been evaluated in a group of healthy subjects.

The screening of the subjects for the intervention study started in September 2009 and continued until month 38 of the project**.**

The preparation of the foods/meals to be given to the participants to the intervention study started in the 1st project period. The schedule for type, time and modalities of sampling, storing and shipping for the blood, urine, faeces and adipose tissue samples to be analysed at the end of the intervention periods was defined after an intensive email communication among the WP5 participants.

Screening, enrolment, and completion of the study during the 1st period: Out of the 50 subjects fulfilling inclusion criteria, 18 was enrolled and randomized to the dietary intervention. Of these, 13 subjects completed the study by month 18. Five subjects were enrolled and completed in month 19. There have been 2 dropouts, one because of moving to another town, one because of serious family health problems. During months 19-54, 65 subjects were enrolled and randomized to the dietary intervention. Out of these, 62 subjects completed the study and 3 were dropouts, because of moving to another town, or serious family health problems. All planned subjects completed the dietary intervention by month 42..

The main significant changes were observed for the groups with high intake of polyphenols. There was number of significantly increased microbial metabolites in the 24h-urinary excretion, indicating the microbial metabolites as compliance biomarkers of polyphenol intake.

Dietary compliance was optimal shown as 7-day food record and as for polyphenols according to the microbial metabolites profile in the urine. Urinary phenolic metabolites increased with high polyphenols diet. Differences with in vitro colon model results likely also reflected polyphenols hepatic metabolism.

The results show trends to differential effects of dietary interventions on postprandial lipoproteins. In particular the diet rich in polyphenols (Figure 8)

- reduced triglyceride-rich lipoproteins both at fasting and in the postprandial period
- reduced HDL cholesterol and triglycerides both at fasting and in the postprandial period
- had no significant effect on fasting and postprandial glucose and insulin levels
- reduced urinary isoprostanes

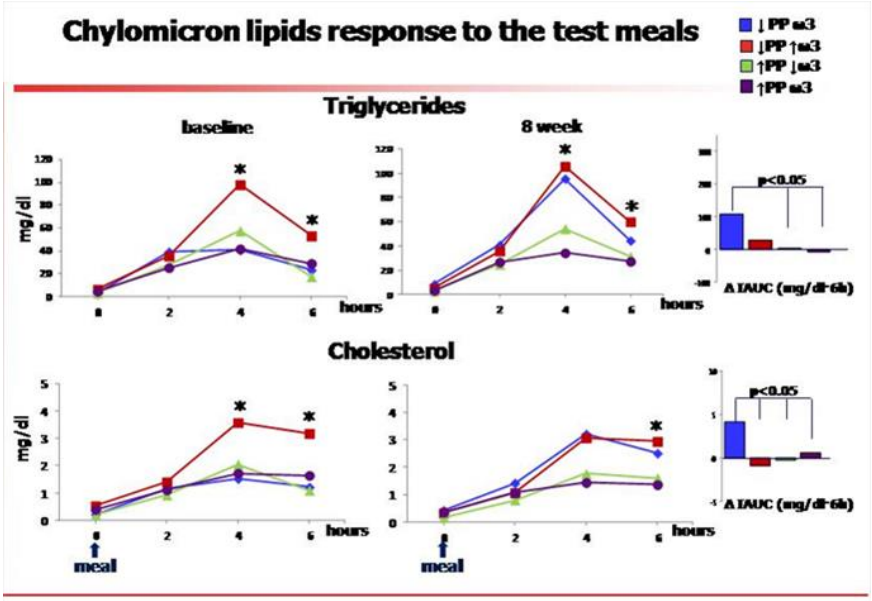


Figure 8 Post-prandial responses of triglyceride-rich lipoproteins: Chlylomicrone lipid response to the test meals: Triglycerides and Cholesterol.

VTT evaluated whether different storage conditions and/or the mostly used DNA-extraction kits affect the Firmicutes and Bacteroides results derived from human faecal samples. The samples were analyzed as fresh and after three different storage temperatures. DNA was extracted using two different, mostly used, commercial kits with 8 different protocols. One kit was based on enzymatic lysis (Qiagen) and the other on mechanical lysis (QBiogene).

The results showed that the mechanical DNA-extraction method to be used for the analysis of the faecal samples of the human nutritional intervention trial to quantify and characterize both Gram-positive and Gram-negative bacteria is as efficient as possible. In addition, the chosen storage temperature (-70°) is optimal. The resulst have been published.

Different diets had different impacts on the faecal bacterial populations. In particular

- The predominant bacterial profiles (Univ-DGGE) were most stable after high consumption of n-3 fatty-acids
- Bifidobacterial profiles (Bif-DGGE) were most unstable after high consumption of polyphenols, The most inter-individual similarity was observed for the Eubacterium rectale group.

- With the diet rich in n-3 fatty acids and polyphenols the most diverse was Bacteroides spp. and the bacterial numbers of the studied bacterial groups increased in over half of the subjects.
- Thus, in the case of fecal microbiota the response to the combination of nutritional substances does not necessarily equal to the cumulative response of singular nutritional substances.

WP6

The initial focus of WP6 was on setting up the infrastructure for the planned analytical developments. The novel time-of-flight mass spectrometric system was installed and testing of the system was performed. In addition the development of new, more efficient methodologies for the characterization of lipid profiles in biological samples was performed. The development was divided into four parts, namely improvement of the global screening method for molecular lipids, development of tools for more accurate identification of unknown molecular species, development of fractionation procedure and development of methodology for the determination of hydrophilic metabolites. During the 1st reporting period, new analytical method was developed for the global screening of the lipid profile and it was utilized in WPs 1-3. For the identification, mass spectra libraries utilising the accurate mass and MS^n fragmentation obtained by the UPLC-Nanomate_LTQ-Orbitrap instrumentation were collected for different sample matrices, including serum and tissue.

During the 2nd reporting period, the fractionation procedure for separation of neutral lipids, glycolipids and phospholipids into individual classes was developed. A UPLC-Nanomate-LTQ-Orbitrap MS instrument was used for structural elucidation of molecular lipids, especially for the characterisation of alkyl-ether and plasmalogen lipids. In addition, the new UPLC-QQQMS instrument was utilised in sensitive analysis of hydrophilic metabolites.

During the 3rd period, the method development for the analysis of hydrophilic metabolites was continued to cover additional classes of endogenous molecules. The improved lipidomics method together with the new lipid library was used for fasted plasma samples of WP 5 human intervention.

New data analysis methods for the treatment of the metabolomics data were developed. For the GC×GC-TOFMS data, an updated version of the software has been developed, allowing for more reliable alignment and normalisation of the data, and as new features, functional group-type identification based on mass spectral fragmentation patterns, as well as several practical features such as search of PubChem and KEGG indexes, features for statistical analyses (PCA, ANOVA, t-test, fold change). The main features of the software are summarised in Figure 9.

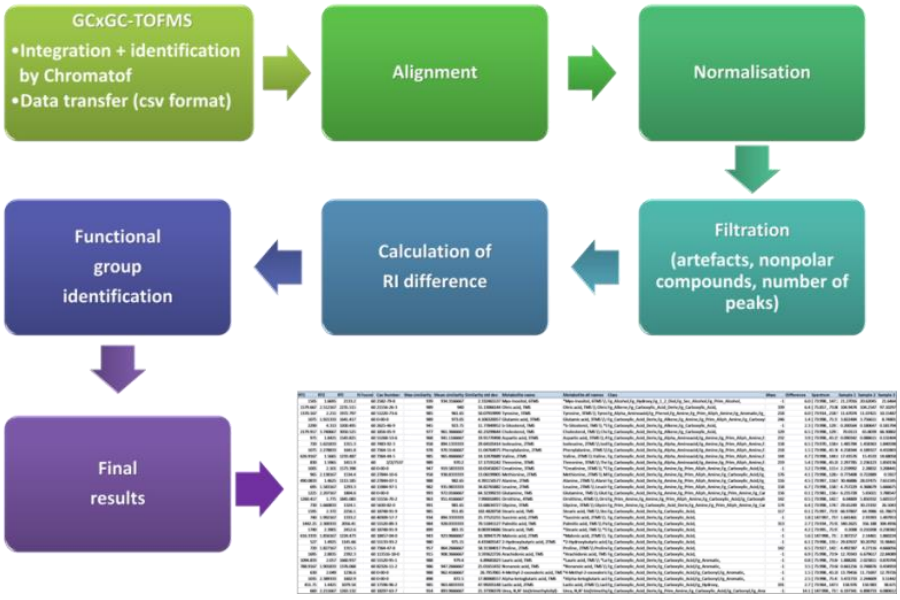


Figure 9 Main features of the Guineu software, developed for the treatment of GC×GC-TOFMS data.

In summary, developments in different research lines as divided into different work packages, were increasingly intertwined as the project progressed. All the main objectives of the project were so met, and both the computational/informatics, analytical tools and protocols were developed which together comprise the nutritional systems biology platform.

Potential impact and main dissemination activities

Impact listed in the call

ETHERPATHS aimed at achieving three main impacts, based on expected impacts listed in the call:

(1) *To improve and strengthen the collaboration between theoretical (dry-lab) and bioanalytical (wet-lab) research.*

ETHERPATHS involved close cooperation of relevant theoretical and bioanalytical research at multiple levels: (A) the *in vitro* studies, focusing on gut microbiota as well as the effect of their metabolic products on host cells, were complemented with modelling of host (adipocyte and hepatocyte) cells, (B) animal model studies were complemented by computational and bioinformatic studies aiming to reconstruct the tissue specific pathways regulated by specific nutritional interventions, and (C) clinical studies with nutritional interventions were complemented with physiological modelling, specifically dynamic models of systemic lipid metabolism. In order to facilitate such dry-lab/wet-lab cooperation in practice, the organization of S/T work packages reflected such a scientific strategy.

In addition, *in silico* modelling was being used to translate the wet-lab research across multiple levels. Specifically, models of systemic lipid metabolism, obtained from human kinetic studies, along with detailed molecular characterization of lipoprotein composition, helped to translate the knowledge obtained from nutritional intervention trial into the human physiological context. As a result, tissue-specific (dys)regulated pathways obtained by pathway reconstruction in specific *in vivo* models were interpreted in the context of human physiology. Furthermore, the cellular models of adipocyte and hepatocyte metabolism were investigated in the context of these pathways. This was made possible by application of *in vitro* colon model and identification and isolation of metabolite pools obtained from nutritional interventions, that was applied to the host cells, thus mimicking the physiological setting. The *meta-model* developed by ETHERPATHS provides a framework for combining models and the data obtained from the models as well as from the bioanalytical platforms across *in vitro*, *in vivo*, and clinical levels.

(2) *To increase the knowledge and give new impetus for health-oriented nutrition research.*

The ETHERPATHS focus on lipid homeostasis and its dietary modulation is of central importance to human health. By using powerful systems biology and bioanalytical tools available to and developed by the ETHERPATHS consortium, new knowledge was generated and disseminated on cellular and systemic metabolism of plasmalogens, on dietary components altering their homeostasis, as well as investigation of potential role of gut microbiota and its metabolites in mediation of plasmalogen metabolism. Such knowledge will be of high importance for health-oriented nutrition research, and will open new possibilities for development of health-oriented nutritional products aiming to maintain or improve lipid homeostasis.

(3) *Computer based tools describing mathematical models of metabolite and energy control is a basic demand for the further development of nutrigenomics and personalized nutrition concepts.*

ETHERPATHS was heavily focusing both on developments of computational models at multiple levels, as well as bridging them with bioanalytical tools providing data for model building as well as for validation. In ETHERPATHS, complex mathematical models at cellular level, focusing on host mammalian cells and enforced by a module for stable isotope tracer data analysis, combined with experimental metabolomic data from the *in vitro* colon model, were complemented by tissue-specific pathway reconstruction methods as part of the *in vivo* studies, and with the dynamic physiological models, focusing on systemic lipid metabolism, *i.e.*, the systemic level of energy control. Unique strength of ETHERPATHS was that these multiple levels were combined both by physiologically motivated models, as well as by practical implementation using powerful software engineering and informatics tools.

Participating SMEs

The project contributed to developments of innovative products and offerings of all three

participating SMEs. Noray Bioinformatics has developed a database system, software and web interface which facilitates construction and use of the mathematical models for nutrition research. Institute for Systems Biology SPb has developed new models for systemic multi-level and multi-compartmental studies of metabolism. The modelling and bioinformatics tools developed by these two SMEs will be incorporated as part of their offering and thus increase their competitiveness. The two participating analytical SMEs (Advion Biosciences, BioMotif) have benefited by further developments of analytical protocols on their platforms, which will lead to new application possibilities and thus potentially to opening new markets for the SMEs.

Wider socio-economic impacts

The development of health claims for food products has been a great challenge for companies in the nutrition sector. One of the difficulties has been assessment of the scientific substantiation of the claims. Ideally, the scientific evidence for any specific claim would include strong clinical evidence for specific health-related (but not medical) outcomes of interest, such as e.g. amount of liver fat, as well as provide a mechanism how a specific food product affects the outcomes of interest, i.e., establish a direct mechanistic link between the food product consumption and the clinical outcome. Such mechanistic insights can only be acquired if one is also studying the physiological effect of foods at different levels, including *in silico*, *in vitro* and *in vivo*. ETHERPATHS has developed a platform for such a purpose and demonstrated it by studying the effects of specific foods on lipid metabolism. However, the platform as such is generic and with problem-specific modification can be applied to study different food products, related to different health-related outcomes.

The model how ETHERPATHS could be applied in nutrition research is shown in Figure 10 below. In short, the health-related outcomes such as specific markers (e.g., the amount of liver fat, which is a known risk factor of diabetes and cardiovascular disease) would be derived from clinical research. A specific food product aimed at affecting the liver fat would then be studied at multiple levels using the ETHERPATHS platform (e.g., by pursuing studies with *in vitro* colon model, hepatocyte and adipocyte cell cultures, suitable animal models, and ultimately a nutritional intervention study in humans – all connected with a suite of tools comprising the ETHERPATHS platform).

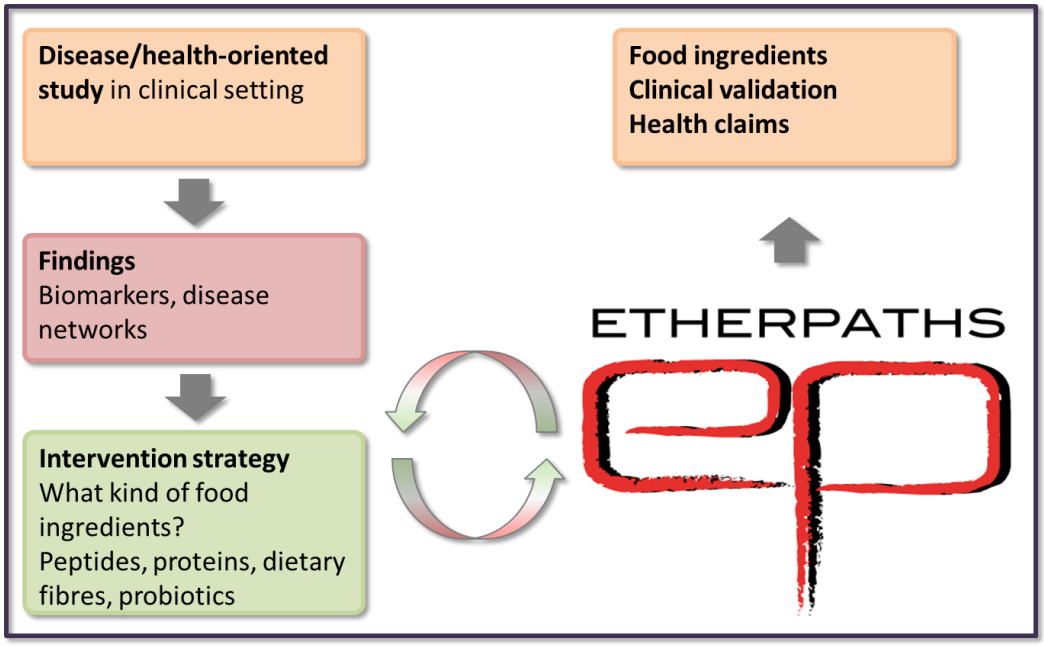


Figure 10 The model how the ETHERPATHS platform would be applied to promote developments of new food products and health claims.

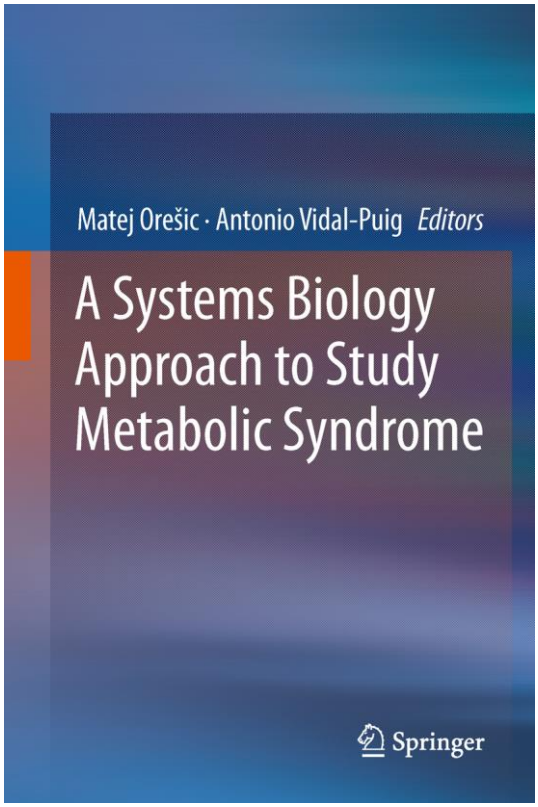
Together, the ETHERPATHS has a potential for economic impact by providing an improved pipeline for health substation of food products, a possibility which will be exploited in the future. Furthermore, use of the ETHERPATHS platform in this setting may ultimately lead to healthier and safer foods, thus leading to public health benefit.

Dissemination

The project participants have been active in publishing scientific papers during the whole project, and as expected these activities have intensified during the last period. Most of the key outcomes of ETHERPATHS are yet to be published. So far, ETHERPATHS papers have occurred in many leading journals in specific domains, including Cell Metabolism, PLoS Biology, PLoS Computational Biology, Gut, Analytical Chemistry, Journal of Nutrition, Bioinformatics, Diabetes, and Diabetes Care, to name a few.

During the last period of the project, the dissemination and exploitation activities have been intensifying. The ETHERPATHS platform has been presented at multiple conferences. For example, there was a special ETHERPATHS session at NuGOweek 2012.

Also a book is released in September 2013 'A Systems Biology Approach to Study Metabolic Syndrome' (Springer Verlag, ISBN 978-3-319-01007-6, eds. Matej Orešič and Antonio Vidal-Puig, ETHERPATHS coordinator and WP2 leader, respectively). The edited volume includes among others also 10 contributions from ETHERPATHS participants, introducing some of the key methodologies and tools comprising the ETHERPATHS platform. The project is specifically acknowledged in the Preface of the book.



4.2 Use and dissemination of foreground

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

Order Nº	Nº	D.O.I.	Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Ope acce is/wil provi to th publica
	27	10.1136/gutjnl-2011-301104	Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88	Larsson, E., Tremaroli, V., Lee, Y.S., Koren, O., Nookaew, I., Fricker, A., Nielsen, J., Ley, R.E., Bäckhed, F.	Gut	61 (2012)	BMJ Publishing Group		23/11/2011	1124-1131	Yes
	28	10.1186/1752-0509-4-135	Edelfosine-induced metabolic changes in cancer cells that precede the overproduction of reactive oxygen species and apoptosis.	Selivanov VA, Vizán P, Mollinedo F, Fan TW, Lee PW, Cascante M	BMC Systems Biology	volume 4	BioMed Central		06/10/2010	135	Yes
	29	10.1007/s11306-011-0328-x	Cyclin-dependent kinases 4 and 6 control tumor progression and direct glucose oxidation in the pentose cycle.	Zanuy M, Ramos-Montoya A, Villacañas O, Canela N, Miranda A, Aguilar E, Agell N, Bachs O, Rubio-Martinez J,	Metabolomics	volume 8	Springer New York		01/06/2012	454-464	Yes

			Pujol MD, Lee WN.P., Marin S, Cascante M.							
	30	10.1371/journal.pcbi.1001115	Reactive Oxygen Species Production by Forward and Reverse Electron Fluxes in the Mitochondrial Respiratory Chain	Selivanov V, Votyakova T, Pivtoraiko V, Zeak J, Sukhomlin T, Trucco M, Roca J, Cascante M	PLoS Computational Biology	volume 7	Public Library of Science	01/03/2011	1001115	Yes
	31	10.1021/jf3052785	Epicatechin gallate impairs colon cancer cell metabolic productivity	Sánchez-Tena S, Alcarraz-Vizán G, Marín S, Torres JL, Cascante M	Journal of Agricultural and Food Chemistry	volume 61	American Chemical Society	08/05/2013	4310-4317	No
	32	10.1152/ajpendo.00675.2011	Target metabolomics revealed complementary roles of hexose- and pentose-phosphates in the regulation of carbohydrate-dependent gene expression	Diaz-Moralli S, Ramos-Montoya A, Marin S, Fernandez-Alvarez A, Casado M, Cascante M	American Journal of Physiology - Endocrinology and Metabolism	volume 303	American Physiological Society	15/07/2012	234-242	No
	33	10.3945/jn.110.133199	A lyophilized red grape pomace containing proanthocyanidin-rich dietary fiber induces genetic and metabolic alterations in colon mucosa of female C57BL/6J mice	Lizarraga D, Vinardell MP, Noé V, van Delft JH, Alcarraz-Vizán G, van Breda SG, Staal Y, Günther UL, Carrigan JB,	Journal of Nutrition	volume 141	American Society for Nutrition	01/09/2011	1597-1604	No

			Reed MA, Ciudad CJ, Torres JL, Cascante M.							
	34	10.1371/journal.pcbi.1002700	Multistationary and oscillatory modes of free radicals generation by the mitochondrial respiratory chain revealed by a bifurcation analysis	Selivanov VA, Cascante M, Friedman M, Schumaker MF, Trucco M, Votyakova TV	PLoS Computational Biology	volume 8	Public Library of Science	01/09/2012	1002700	Yes
	35		Kinetic modeling as a tool to integrate multilevel dynamic experimental data.	1. Mogilevskaya E, Bagrova N, Plyusnina T, Gizzatkulov N, Metelkin E, Goryacheva E, Smirnov S, Kosinsky Y, Dorodnov A, Peskov K, Karelina T, Goryanin I, Demin O.	Methods in Molecular Biology	2009, 563	Humana Press	31/12/2009	197-218	No
	36	10.1111/j.1742-4658.2009.07394.x	Modelling of ATP-ADP steady-state exchange rate mediated by the adenine nucleotide translocase in isolated mitochondria	Metelkin, E., Demin, O., Kovacs, Z., Chinopoulos, C. 2009.	FEBS Journal	2009, 276	Blackwell Publishing	26/10/2009	6942-6955	No
	37	10.111/j.1742-4658.2012.08719x	Kinetic modelling of central carbon metabolim in Escherichia coli.	Peskov, K., Mogilevskaya, E. and Demin, O.	FEBS Journal	2012, 279	Blackwell Publishing	03/09/2012	3374-3385	Yes
	38		DBSolve Otimum: a software package for kinetic modelling which allows dynamic visualization of	Gizzatkulov, N.M., Goryanin,	BMC Systems Biology	4 (2010)	BioMed Central	10/08/2010	109-119	Yes

		simulation results	I.I., Metelkin, E.A., Mogilevskaya, E.A., Peskov, K.V. and Demin, O.V.						
	10.1111/j.1574-6941.2011.01257.x	The currently used commercial DNA-extraction methods give different results of clostridial and actinobacterial populations derived from human fecal samples	Johanna Maukonen	FEMS Microbiol Ecol	79 (2012)	Blackwell Publishing Ltd	13/12/2011	697-708	Yes
	10.1161/ATVBAHA.111.224808	Dual metabolic defects are required to produce hypertriglyceridemia in obese subjects.	Jan Boren	Arterioscler Thromb Vasc Biol.	2011 Sep;31(9)		21/07/2011	2144-50	No
	10.1002/rcm.4422	Optimization of N-methyl-N-[tert-butyldimethylsilyl]trifluoroacetamide as a derivatization agent for determining isotopic enrichment of glycerol in very-low density lipoproteins	Barbara Fielding	Rapid Commun Mass Spectrom	Volume 24, Issue 5, pages , 15 March 2010		15/03/2010	586–592	No
	10.1111/j.1365-2796.2010.02292.x	LDL-associated apolipoprotein J and lysozyme are associated with atherogenic properties of LDL found in type 2 diabetes and the metabolic syndrome	Linda Fogelstrand	Journal of internal medicine	Volume 269, Issue 3		17/02/2011	306–321	No
	10.2337/db09-0206	ApoCIII-enriched LDL in type 2 diabetes displays altered lipid composition, increased susceptibility for sphingomyelinase, and increased binding to biglycan.	Jan Borén	Diabetes	58(9)		05/06/2009	2018-26	Yes
	10.1242/dmm.009845	Obesity and psychotic disorders: uncovering common mechanisms through metabolomics	Matej Oresic	DMM Disease Models and Mechanisms	5(5)	Company of Biologists Ltd	29/09/2012	614-620	Yes

	10.1007/s00394-012-0391-8	Characterization of microbial metabolism of Syrah grape products in an in vitro colon model using targeted and non-targeted analytical approaches	Anna-Marja Aura	European Journal of Nutrition	25th May 2012	D. Steinkopff-Verlag		16/06/2012	1-14	Yes
	10.1016/j.cmet.2013.01.003	Gut Microbiota Regulates Bile Acid Metabolism by Reducing the Levels of Tauro-beta-muricholic Acid, a Naturally occurring FXR Antagonist	S.I. Sayin, A. Wahlström, J. Felin, S. Jäntti, H.-U. Marschall, K. Bamberg, B. Angelin, T. Hyötyläinen, M. Oresic, Fredrik Bäckhed	Cell Metabolism	17 (2013)	Cell Press		05/02/2013	225-235	No
		Bistability of mitochondrial respiration underlies paradoxical reactive oxygen species generation in	Marta Cascante	PLoS computational biology	Volume 5, issue 12	Public Library of Science	United States	24/12/2009	electronic e1000619	Yes
		Histone deacetylase inhibition results in a common metabolic profile associated with HT29 differenti	Marta Cascante	Metabolomics : Official journal of the Metabolomic Society	volume 6, issue 2	New York : Springer, c2006-	United States	08/01/2010	229-237	Yes
		Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activ	Marta Cascante	Journal of immunology	volume 185, issue 1	American Association of Immunologists	United States	24/05/2010	605-614	No
		MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molec	Matej Oresic	BMC Bioinformatics	Vol 11: 395	BioMed Central Ltd	United Kingdom	23/07/2010	395	Yes
		Composition and lipid spatial distribution of HDL particles in	Matej Oresic	J. Lipid Res.	Volume 51	American Soc. for Biochem.	United States	27/04/2010	2341-2351	No

		subjects with low and high HDL-choles				Mol. Biol. Inc					
☐		MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data	Matej Oresic	BMC Bioinfromatics	11 (2010)	BioMed Central Ltd		23/07/2010	395-405	Yes
☐	10.1016/j.atherosclerosis.2012.02.001	Postprandial accumulation of chylomicrons and chylomicron remnants is determined by the clearance capacity	Marja-Riitta Taskinen	Atherosclerosis		Elsevier				No
☐	10.1093/imammb/dqr021	Investigations of a compartmental model for leucine kinetics using non-linear mixed effects models with ordinary and stochastic differential equations.	Bernt Wennberg	Mathematical Medicine and Biology		Oxford Journals				No
☐	10.1194/jlr.M002774	The gut microbiota modulates host energy and lipid metabolism in mice	Bäckhed, F.	Journal of Lipid Research	51 (2010)	American Society for Biochemistry and Molecular Biology Inc.		29/12/2009	1101-1112	Yes
☐		An allostatic control of membrane lipid composition by SREBP1.	Hagen RM, Rodriguez-Cuenca S, Vidal-Puig A.	FEBS Lett.	584(12)	Elsevier	Netherlands	18/06/2010	:2689-98	No
☐		Origins of metabolic complications in obesity: ectopic fat accumulation. The importance of the qualitative aspect of lipotoxicity.	Carobbio S, Rodriguez-Cuenca S, Vidal-Puig A.	Curr Opin Clin Nutr Metab Care	14(6)	LIPPINCOTT WILLIAMS & WILKINS	UNITED STATES	01/11/2011	520-6.	No
☐	10.1194/jlr.M006494	Composition and lipid spatial distribution of HDL particles in subjects with low and high HDL-cholesterol	Matej Orešič	Journal of Lipid Research	51 (2010)	American Society for Biochemistry and Molecular Biology, Inc.		27/04/2010	2341-2351	Yes

	10.1186/1752-0509-5-175	Compartmentation of glycogen metabolism revealed from 13C isotopologue distributions	Marta Cascante	BMC Systems Biology	5 (2011)	BioMed Central		28/10/2011	175	Yes
	10.1016/j.bbalip.2011.06.012	Review Informatics and computational strategies for the study of lipids	Matej Orešič	Biochimica et Biophysica Acta	1811 (2011)	Elsevier B.V.		25/06/2011	991-999	Yes
	10.1093/bioinformatics/btr278	MPEA—metabolite pathway enrichment analysis	Matej Orešic	Bioinformatics	27 (2011)	Oxford University Press		05/05/2011	1878-1879	Yes
	10.1016/j.bbalip.2009.11.003	Review: Systems biology strategy to study lipotoxicity and the metabolic syndrome	Matej Oresic	Biochimica et Biophysica Acta	1801 (2010)	Elsevier		26/11/2009	235-239	Yes
	10.1186/1752-0509-5-175	Compartmentation of glycogen metabolism revealed from 13C isotopologue distributions	Marta Cascante	BMC Systems Biology	Volume 5: 175	BioMed Central Ltd	United Kingdom	28/10/2011	175	Yes
	10.1021/ac103308x	Data Analysis Tool for Comprehensive Two-Dimensional Gas Chromatography/Time-of-Flight Mass Spectrometry	Tuulia Hyötyläinen	Anal. Chem.	83 (2011)	ACS Publications		24/03/2011	3058-3067	Yes

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
1	Press releases	VALTION TEKNILLINEN TUTKIMUSKESKUS	Project launched by VTT develops computer models for predicting dietary effects on	11/03/2009	VTT Public web	Scientific community (higher education, Research)	9999	Finland, Europe	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
			health			- Industry - Policy makers - Medias					
2	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	ETHERPATHS project develops computer models for predicting dietary effects on health	01/09/2009	NugoWeek2009, Montecatini Terme, Italy	Scientific community (higher education, Research)	300	Europe, USA etc	VALIDATED	update delete	
3	Organisation of Workshops	GOETEBORGS UNIVERSITET	EtherPaths Mini-Symposium on nutritional systems biology	28/03/2011	The Swedish Exhibition and Congress Centre (Confreence room R2)	Scientific community (higher education, Research)	50	Sweden	VALIDATED	update delete	
4	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Lipidome in health and disease - an allostatic perspective	28/09/2011	Helsinki, Finland	Scientific community (higher education, Research) – Industry	230	China, Australia, United States, Europe	VALIDATED	update delete	
5	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Phenolic nutrional phytochamicals as biomarkers of plant food intake	29/09/2011	Giovinnazzo (Bari) Italy	Scientific community (higher education, Research) - Industry - Policy makers	120	Australia, Europe, India, Nigeria, Singapore, South Korea, Thailand	VALIDATED	update delete	
6	Presentation	VALTION TEKNILLINEN	Lipidome in Health and Disease - An	20/06/201	Stockholm, Sweden	Scientific communit	300	Sweden, Europe, Asia,	VALIDATE	update	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
	s	TUTKIMUSKESKUS	Allostatic Perspective	1		y (higher education, Research) - Industry		America	D	delete	
7	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Adipose tissue lipidome: benefits and costs of lipid remodeling as adaptation to acquired obesity	24/08/2011	Bangalore, India	Scientific community (higher education, Research) - Industry	150	Asia	VALIDATED	update delete	
8	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Lipidome in health and metabolic disease	24/10/2011	Erice, Italy	Scientific community (higher education, Research) - Industry	250	Europe, USA, Asia	VALIDATED	update delete	
9	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Diet- and drug-related intestinal metabolome	12/11/2010	The 4th Finnish Gut Day, Helsinki, Finland	Scientific community (higher education, Research) - Industry	91	Finland, UK, Belgium, Schwitzerland	VALIDATED	update delete	
10	Presentations	VALTION TEKNILLINEN TUTKIMUSKESKUS	Systems biology in food and nutrition research	24/03/2011	ChemBio-Finland 22.-24.3.2011, Helsinki, Finalnd	Scientific community (higher education, Research) - Industry - Medias	300	Finland	VALIDATED	update delete	
11	Presentations	UNIVERSITAT DE BARCELONA	Metabolic basis of complex diseases	18/02/2009	Barcelona School on Biomedical Informatics, Barcelona, Spain	Scientific community (higher	100	Europe	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
						education, Research)					
12	Presentations	UNIVERSITAT DE BARCELONA	The robust Metabolic Network Adaptations in Multifactorial Diseases	05/11/2009	Advances in Metabolomic Profiling, European Biomarkers Summit, Proteomics Europe.., Barcelona, Spain	Scientific community (higher education, Research)	500	Europe	VALIDATED	update delete	
13	Presentations	UNIVERSITAT DE BARCELONA	The robust Metabolic Network Adaptations in Multifactorial Diseases	26/11/2009	III Jornadas de Modelización y simulación en Biomedicina; Barcelona, Spain	Scientific community (higher education, Research)	100	Spain	VALIDATED	update delete	
14	Presentations	UNIVERSITAT DE BARCELONA	Systems Biology in mutifactorial diseases.	14/12/2009	5th Meeting of the Spanish Network of Systems Biology (REBS), Madrid, Spain	Scientific community (higher education, Research)	150	Spain	VALIDATED	update delete	
15	Presentations	UNIVERSITAT DE BARCELONA	Metabolic Alteration underlying multi-factorial diseases as target for novel therapies	20/01/2010	Integrative Biology Workshop, Birmingham, UK	Scientific community (higher education, Research)	50	Europe	VALIDATED	update delete	
16	Presentations	UNIVERSITAT DE BARCELONA	Metabolic Alterations as Targets for Novel Therapies	11/10/2010	11th Internacional Conference on Systems Biology. ICSB 2010; Edimburg, UK	Scientific community (higher education, Research)	800	World	VALIDATED	update delete	
17	Presentation	UNIVERSITAT DE	The metabolic alterations as new	09/10/201	Integrative OMICS approaches to disease mechanisms-	Scientific communit	100	Europe	VALIDATE	update	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
	s	BARCELONA	targets in the design of therapies for multifactorial diseases	1	Freiburg, Germany	y (higher education, Research)			D	delete	
18	Posters	UNIVERSITAT DE BARCELONA	Characterization of the metabolic fluxes in the adipocyte differentiation process using bioinformati	12/09/2011	47th annual meeting European association for the study of diabetes, Lisbon, Portugal	Scientific community (higher education, Research)	9999	Europe	VALIDATED	update delete	
19	Presentations	UNIVERSITAT DE BARCELONA	Teaching Systems Biology to Master Students	28/08/2011	ICSB 2011 Workshop on Challenges in Future Education and Training, Heidelberg, Germany	Scientific community (higher education, Research)	50	Europe	VALIDATED	update delete	
20	Presentations	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Introduction to the EtherPaths Special Session at NuGOweek 2012	29/08/2012	Helsinki, Finland	Scientific community (higher education, Research) - Industry - Civil society	164	Europe, Australia, Israel, Brazil, New Zealand, USA	VALIDATED	update delete	
21	Posters	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Colonic metabolites of polyphenol-rich foods as a compliance biomarker of the intake	18/01/2013	Helsinki, Finland	Scientific community (higher education, Research) - Industry	78	Finland, The Netherlands, Ireland	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
22	Articles published in the popular press	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Gut bacteria like food, which is rich in dietary fibre. (In Finnish)	31/03/2012	Kehittyvä elintarvike-lehti (trade journal)	Scientific community (higher education, Research) - Industry - Civil society	2500	Finland	VALIDATED	update delete	
23	Articles published in the popular press	TEKNOLOGIAN TUTKIMUSKESKUS VTT	You are what your bacteria eat (In Finnish)	31/03/2012	Tiede-lehti, p.14-15	Scientific community (higher education, Research) - Industry - Medias	60951	Finland	VALIDATED	update delete	
24	Articles published in the popular press	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Fibre protects from cancer (In Finnish)	31/10/2011	Kotiliesi-lehti p. 76	Civil society - Medias	119105	Finland	VALIDATED	update delete	
25	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	"Ether-lipids (Plasmalogens): A possible role in insulin resistance/obesity ?.	17/11/2009	Institute of Metabolic Sciences. Cambridge	Scientific community (higher education, Research)	50	UK	VALIDATED	update delete	
26	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	The role of plasmalogens in the Metabolic Syndrome.	19/07/2010	IMS PhD student symposium	Scientific community (higher education, Research)		UK	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
27	Posters	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Are Plasmalogens novel players in the Metabolic Syndrome?	04/10/2010	IMS away day.Wellcome Trust Conference Centre at Hinxton	Scientific community (higher education, Research)	100	UK	VALIDATED	update delete	
28	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	The role of plasmalogens in the Metabolic Syndrome	26/09/2011	IMS PhD student symposium	Scientific community (higher education, Research)		UK	VALIDATED	update delete	
29	Posters	UNIVERSITAT DE BARCELONA	Analysis of the control exerted by glucokinase and glucose-6-phosphatase on glycerol metabolism of l	20/09/2010	46th EASD Annual Meeting, Stockholm, Sweden	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
30	Posters	UNIVERSITAT DE BARCELONA	Characterisation of the metabolic fluxes in the adipocyte differentiation process using bioinformati	05/09/2011	XXXIV Congreso de la Sociedad Española de Bioquímica, Barcelona, Spain	Scientific community (higher education, Research)		Spain	VALIDATED	update delete	
31	Oral presentation to a scientific event	UNIVERSITAT DE BARCELONA	Relationships between oxygen transport and ROS generation	13/06/2012	EISBM Workshop on Knowledge Management for Translational Research, Lyon, France	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
32	Oral presentation to a scientific event	UNIVERSITAT DE BARCELONA	Understanding and Targeting Metabolism for Drug Discovery in Multifactorial Diseases	25/06/2012	8th International Conference of the Metabolomics Society, Washington,D.C, USA	Scientific community (higher education, Research)		World	VALIDATED	update delete	
33	Posters	UNIVERSITAT DE BARCELONA	Targeting Metabolism as a Therapeutic Approach for Multifactorial Diseases	06/07/2012	Cell Symposia: Angiogenesis, Metabolic Regulation, and Cancer Biology in association with VIB, Leuve	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
34	Oral presentation to a scientific event	UNIVERSITAT DE BARCELONA	Metabolic modelling for nutrition research	28/08/2012	NuGOweek 2012 symposium: Nutrition, lifestyle and genes in the changing environment, Helsinki, Finla	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
35	Posters	UNIVERSITAT DE BARCELONA	Targeting Metabolism as a Therapeutic Approach for Obesity and Multifactorial Diseases	13/09/2012	EMBO/EMBL Symposium: Diabetes and Obesity, Heidelberg, Germany	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
36	Posters	UNIVERSITAT DE BARCELONA	Characterization of the metabolic fluxes in the adipocyte differentiation process using bioinformati	13/09/2012	EMBO/EMBL Symposium: Diabetes and Obesity, Heidelberg, Germany	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
37	Oral presentation to a scientific event	UNIVERSITAT DE BARCELONA	Estimation of metabolic fluxes in health and disease by tracer based metabolomics	21/02/2013	EASL Monothematic Conference: Systems biology of the liver, Luxembourg	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
38	Posters	UNIVERSITAT DE BARCELONA	Use of bioinformatics tools for fluxomics analysis of the metabolic changes during adipocyte differe	01/07/2013	9th Annual Conference of the Metabolomics Society, Glasgow, United Kingdom	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	
39	Oral presentation to a scientific event	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Regulation of lipid metabolism by gut microbiota and dietary lipids	30/08/2012	NuGOWeek 2012 Nutrition, lifestyle and genes in the changing environment, Helsinki, Finland	Scientific community (higher education, Research) - Industry	164	World	VALIDATED	update delete	
40	Articles published in the popular press	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Ultra high performance liquid chromatography-mass spectrometry in lipidomics	01/03/2013	Peer-reviewed article in LCGC trade magazine. Open access.	Scientific community (higher education, Research) - Industry - Medias	10000	Europe	VALIDATED	update delete	
41	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	"Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome	06/12/2011	Nestle Institute Health Research, Lausanne, Switzerland	Scientific community (higher education, Research)		Europe	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
42	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	"Adipotoxicity and metabolic syndrome".	26/03/2010	Kyoto, Japan	Scientific community (higher education, Research)		Japan	VALIDATED	update delete	
43	Interviews	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	healthy obesity	01/03/2012	Basque Country Radio (Radio Euskadi)	Civil society		Spain	VALIDATED	update delete	
44	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	The concept of healthy obesity	01/03/2012	CIC bioGUNE | Fundación BBVA (Bilbao, Spain)	Scientific community (higher education, Research) - Medias		Spain	VALIDATED	update delete	
45	Articles published in the popular press	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Overweight and Healthy Obesity	24/07/2011	http://www.finanzas.com webpage	Civil society		Spain	VALIDATED	update delete	
46	Posters	TEKNOLOGIAN TUTKIMUSKESKUS VTT	Effect of nutritional intervention and etherlipid depletion on lipid metabolism in animal model.	01/07/2013	Metabolomcs 2013, Glasgow, Uk	Scientific community (higher education, Research)	700	World	VALIDATED	update delete	
47	Oral	TEKNOLOGIAN	Phenolic	29/08/201	NuGOWeek2012, Nutrition,	Scientific	164	Europe,	VALIDATE	update	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
	presentation to a scientific event	TUTKIMUSKESKUS VTT	nutritional phytochemicals as biomarkers of plant intake?	2	lifestyle and genes in the Changing Environment	community (higher education, Research) - Industry - Medias		Australia, New Zealand, Israel,Brazil, USA	D	delete	
48	Press releases	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Obesity Epidemy	11/06/2013	http://www.informavalencia.com/	Civil society		Spain	PENDING	update validate reject	
49	Press releases	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Overweight and Healthy Obesity	14/01/2013	www.diariomedico.com	Scientific community (higher education, Research)		Spain	PENDING	update validate reject	
50	Press releases	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Tsunami of Obesity	30/11/2012	http://www.fundacionpunset.org/	Civil society		Spain	PENDING	update validate reject	
51	Articles published in the popular press	Institute for Systems Biology SPb Company Limited	EI of Phosphotransferase System of Escherichia coli: Mathematical modelling approach to analysis of	20/01/2011	J Biophysics Hidawi Open access Trade journal	Scientific community (higher education, Research) - Industry	10000	World	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
52	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Adipose tissue expandability, lipotoxicity and the Metabolic syndrome	13/09/2012	EMBL Heidelberg, Germany	Scientific community (higher education, Research)		Europe	PENDING	update validate reject	
53	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Adipose Tissue Expandability, Lipotoxicity and the Metabolic Syndrome	30/08/2012	BENZON SYMPOSIUM No. 58 Adipose Tissue in Health and Disease, Copenhagen	Scientific community (higher education, Research)		Europe	PENDING	update validate reject	
54	Oral presentation to a scientific event	THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF CAMBRIDGE	Adipose Tissue Expandability, Lipotoxicity and the Metabolic Syndrome	08/03/2013	2nd International Congress on Translational Research in Human Nutrition. (Clermont-Ferrand, France)	Scientific community (higher education, Research)		Europe	PENDING	update validate reject	
55	Posters	Institute for Systems Biology SPb Company Limited	Circulator Is a New Technique for Systems Pharmacology	08/06/2010	PAGE meeting 2010, Berlin, Germany	Scientific community (higher education, Research)		World	VALIDATED	update delete	
56	Posters	Institute for Systems Biology SPb Company Limited	Modeling of evolution of bacterial community composition depending on ability of different bacteria	28/08/2011	International Conference on Systems Biology 2011, Heidelberg, Germany	Scientific community (higher education, Research)	1500	Europe, USA	VALIDATED	update delete	

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Status	Actions	Order
57	Oral presentation to a scientific event	Institute for Systems Biology SPb Company Limited	Modeling in Systems Biology: applications in biotechnology and biomedicine	12/04/2010	EurasiaBio Conference 2010, 12-15 April, 2010, Moscow, Russia	Scientific community (higher education, Research)	500	Russia, Europe, USA	VALIDATED	update delete	
58	Oral presentation to a scientific event	Institute for Systems Biology SPb Company Limited	Systems pharmacology modellingin drug research and development	22/04/2012	BioITWorld Conference 2012, Boston, 22-26 April 2012,	Scientific community (higher education, Research)	2000	Europe, USA, China, India, Russia	VALIDATED	update delete	

Section B (Confidential[2] or public: confidential information to be marked clearly)
Part B1

No patents during the project duration. Main focus has been on platform developments, which are not directly patentable.

Part B2
Project Exploitable Foregrounds

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved	Status	Actions
Commercial exploitation of R&D results	Method for preparation of colon model extracts for mammalian cell models	Yes		Purified extract containing microbial metabolome of a food product	Industrial	01/01/2014	Utility model considered	VTT B1b	VALIDATED	update delete
General advancement of knowledge	Nutritional Systems Biology Platform	No		Use of food microbial metabolite extracts on cell models	Research	01/06/2014	Publishable	B1, B2, B3b and B4	VALIDATED	update delete
General advancement of knowledge	Metabolite profiling and data analysis of microbial metabolites from food and beverage products	No		Identification of microbial metabolite profiles available for circulation in human body	Research Industrial	01/06/2013	Publishable	Beneficiary B1b	VALIDATED	update delete
General advancement of knowledge	New protocol and laboratory procedures for	Yes		New study design, new laboratory analyses	Research	First study to be completed in 2014	None	B3b	VALIDATED	update delete

[2] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved	Status	Actions
	non-steady state kinetics									
General advancement of knowledge	New computational model for non-steady state kinetics	Yes		New computational model and implementation	Research	First study to be completed in 2014	None	B3b	VALIDATED	update delete
General advancement of knowledge	SBML model design and management tool	Yes		New computional tool	Research	30/06/2013	none	NorayBio B7	VALIDATED	update delete

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

Method for preparation of colon model extracts for mammalian cell models
The purpose of the method is to prepare microbial metabolome from an authentic food as purified, which mimicks the metabolites reaching peripheral tissue in humans to verify the mecahnisms related to those metabolites formed from the food. Companies may be interested if their prooducts would caus certain helath benfits, which can be detected from cell models, essential for verification of a health claim in connection with a clinical output. The schedule could be in 2014-2015 after dissemination of the availability. The method could be protected by a utility model and this will be considered. The further research is related to the cell models, which type of responses can be performed. Possible impact could be finding out e.g. foods causing a metabolome affecting adiopocyte health and triggerring type 2 diabetes.

Nutritional Systems Biology Platform
Nutritional Systems Biology Platform combines the colon model and its extract preparation from genuine food products or ingredients with application of pure extracts to cell models to study biological effects. This can be exploited by any food or ingredient company who wishes to take into account the microbial metabolism of their food (phyto)chemicals. This platform has already been introduced to companies and can be offered as a service for them. Colon model and extract preparation are already a routine, but each food matrix and mammalian cell assay requires its own applications and conditions to ensure authentic and as physiological application and dosage as possible. This platform can be used in research projects or contract research with genuine products to verify the mechanisms of food metabolites on mammalian cells e.g. in collecting health claim dossiers.

Metabolite profiling and data-analysis of the microbial metabolites from food and beverage products
Plant foods are in principal a source of dietary fibre, which is detined to colon and the conversion activities of its microbiota. The diversity of phytochemicals in the DF of plant foods is beyond those quantitated routinely and the compound pool is structurally modified by the action of the microbiota. The converted microbial metabolites are those which appear in urine and also in plasma as measurable quantities and thus it is important to identify the compounds formed

in different foods. After identification, the compounds can be used as compliance biomarkers of plant food intake. The impact of metabolite profiling is to show components which are circulating in human body instead of asking the food intake form dietary daily records. The identification also helps in understanding mechanisms of action, when the compounds can be introduced to mechanistic designs for verification. Offerring can be for industry or for research purposes. The methods have been published.

New protocol and laboratory procedures for non-steady state kinetics and
New computational model for non-steady state kinetics
The experimental and computational procedures will allow more accurate characterization of systemic lipid metabolism based on the outcomes of human tracer studies. Technologies for the human tracer studies are a niche domain in clinical research, of potential interest to companies in medical device/diagnostic sectors.

SBML model design and management tool
The tool facilitate building and implementation of computational models of biological systems using the Systems Biology Markup Language (SBML). This tool has been implemented by NorayBio as part of bioinformatics software package for managing data and models as related to nutritional systems biology studies and is thus already exploited as a product.

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**FP-7-KBBE-222639-2**
Title of Project:	**Characterization and modelling of dietary effects mediated by gut microbiota on lipid metabolism**
Name and Title of Coordinator:	**Matej Orešič, Research Professor, VTT, Valtion teknillinen tutkimuskeskus**

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	●***Yes 0No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	●
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	●
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	●
• Did the project involve Human data collection?	●
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual	

lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	●
• Were those animals transgenic small laboratory animals?	●
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes ●No
• Research having the potential for terrorist abuse	0 Yes ●No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	1
Work package leaders	3	6
Experienced researchers (i.e. PhD holders)	19	24
PhD Students	9	3
Other	59	19

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**13**
Of which, indicate the number of men:	9

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

● Yes
○ No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
●	Design and implement an equal opportunity policy	○	○	○	○	●
●	Set targets to achieve a gender balance in the workforce	○	○	○	○	●
❑	Organise conferences and workshops on gender	○	○	○	○	○
●	Actions to improve work-life balance	○	○	○	●	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

● Yes- please specify: Both genders were included in the intervention trial.

○ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

● Yes- please specify Explanation of the project to College students as part of PhD programme dissemination activity (UCAM), PhD Students (UB)

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

● No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

● Main discipline[3]: 2.3

● Associated discipline[3]: 3.1

● Associated discipline[3]: 1.5

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

● Yes
○ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

● No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

[3] Insert number from list below (Frascati Manual).

○ Yes, in communicating /disseminating / using the results of the project		
11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ●	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ● No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ● Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	●
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ● International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**64**
To how many of these is open access[4] provided?	**22**
How many of these are published in open access journals?	**22**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	**42**
Please check all applicable reasons for not providing open access:	
● publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ● no suitable open access journal available ● no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[5]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		**0**

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

● Increase in employment, or	● In small & medium-sized enterprises
❑ Safeguard employment, or	❑ In large companies
❑ Decrease in employment,	❑ None of the above / not relevant to the project
❑ Difficult to estimate / not possible to quantify	

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	**11**

[4] Open Access is defined as free of charge access for anyone via Internet.
[5] For instance: classification for security project.

Difficult to estimate / not possible to quantify ❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ● No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

● Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

●	Press Release	●	Coverage in specialist press
❑	Media briefing	●	Coverage in general (non-specialist) press
❑	TV coverage / report	●	Coverage in national press
●	Radio coverage / report	●	Coverage in international press
●	Brochures /posters / flyers	●	Website for the general public / internet
❑	DVD /Film /Multimedia	●	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	●	English
●	Other language(s) Spanish		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

2. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1.	
2.	
n	
Total	